ISOMERIZATION OF D-FRUCTOSE BY BASE: LIQUID-CHROMATOGRAPHIC EVALUATION AND THE ISOLATION OF D-PSICOSE*

LANDIS W. DONER
Eastern Regional Research Center†, Philadelphia, Pennsylvania 19118 (U.S.A.)

ABSTRACT

Several bases have been evaluated as catalysts for the production of D-psicose (D-*ribo*-2-hexulose) from D-fructose. The hexose levels in the isomerized mixtures were quantified by l.c. on a μBondapak/Carbohydrate column. The most effective and convenient base was found to be pyridine, and mixtures produced by boiling concentrated solutions (1 g/mL) of D-fructose in pyridine under reflux contained 12.4% of psicose, lesser proportions of glucose and mannose, and 25.8% of the starting material. Following removal of solvent, fermentation with bakers' yeast removed most hexoses other than D-psicose, which was isolated by chromatography on cellulose. The entire procedure required three days, and D-psicose was obtained in gram quantities in 6.8% of the theoretical yield.

INTRODUCTION

D-Psicose (D-*ribo*-2-hexulose) was first identified[1] as a nonfermentable constituent of cane molasses, and was presumed to have been formed by a Lobry de Bruyn–Alberda van Ekenstein transformation. The sugar was then found to occur naturally as the sugar moiety of the nucleoside antibiotic psicofuranine[2,3], and later to exist as the free sugar in wheat[4] and *Itea* plants[5]. Human urine contains 15–30 mg/L, which presumably originates in the diet[6]. Recently, the fate of radioactive psicose after oral and intravenous administration to rats has been examined[7]. Whereas most of the radioactivity rapidly appeared in the urine as unchanged psicose when the sugar was administered intravenously, more than one-third of the radioactivity was retained by the carcass, and 15% was exhaled as carbon dioxide after oral feeding. It was suggested that a large proportion of the ingested psicose is metabolized by intestinal flora.

Psicose is not known crystalline, possibly because no single ring-form preponderates. Aqueous solutions of psicose at 30° contain the α-furanose, β-furanose,

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†Agricultural Research, Science, and Education Administration, U.S. Department of Agriculture.

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α-pyranose, and β-pyranose forms in the ratio of 38:15:26:21, based on relative integration-areas of the anomeric-carbon atom signal in the ^{13}C-n.m.r. spectrum[8].

Numerous preparatory methods for psicose have been described, some utilizing bases to isomerize D-allose[9], D-glucose[10], or D-fructose[11], whereas others have involved multistep, synthetic schemes. In the example of D-fructose isomerization[11], *N,N*-dicyclohexocarbodiimide was employed as base, and a small quantity of psicose was isolated by preparative paper-chromatography after the mixture had been fermented with yeast. The synthetic method for the production of psicose in larger quantities is the modification by Tipson *et al.*[12] of McDonald's[13] 4-step scheme beginning with D-fructose. In this procedure, 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose was oxidized to the 3-keto derivative, and then stereospecific reduction with sodium borohydride gave 1,2:4,5-di-*O*-isopropylidene-β-D-psicopyranose; removal of the isopropylidene groups, afforded D-psicose.

Although the method of Tipson *et al.*[12] is wholly adequate for the large-scale preparation of D-psicose, such procedures often consume inordinate amounts of time, especially if the sugar is required as a standard. The present research was undertaken to establish a rapid method for preparing gram quantities of D-psicose, as this sugar is not commercially available. D-Psicose was required as a standard for our research program aimed at detection of honey and high-fructose corn syrup in admixture.

RESULTS AND DISCUSSION

The l.c. separation of a standard mixture of the sugars used in this study is shown in Fig. 1. As indicated, the separation requires less than 15 min; ketoses are eluted prior to aldoses, and psicose is effectively separated from fructose. Mixtures resulting from isomerization of fructose were conveniently quantified by comparing peak heights with those of a known amount of the corresponding sugar in the standard mixture. Mannitol was included in the standard mixture in Fig. 1 because it is known to result from fermentation of D-fructose by yeast.

Table I summarizes the chromatographic behavior of the standard sugars on the μBondapak/Carbohydrate column. The ketoses and mannitol are appreciably more responsive to refractive-index detection than are the aldoses. The same order of response has been observed in this laboratory with far-ultraviolet detection at 195 nm, although the procedure is much less sensitive than the refractive-index detector.

Liquid-chromatographic evaluation of the capacity of various bases to isomerize D-fructose to D-psicose has shown that pyridine is the most effective, and fewer operations are required in the processing of reaction-mixtures employing this base. Table II summarizes the yields of hexose obtained by isomerization of D-fructose with the various bases. In all instances, much of the D-fructose consumed is not accounted for by the hexose totals. It may be expected that, in addition to isomerization, rearrangement to saccharinic acids and reverse-aldol fragmentations occur.

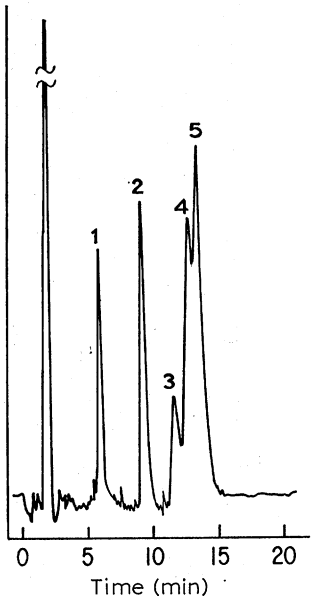

Fig. 1. Liquid-chromatographic separation of the standard psicose (**1**), fructose (**2**), mannose (**3**), glucose (**4**), and mannitol (**5**). The μBondopak/Carbohydrate column was eluted with 88:12 (w/w) acetonitrile–water, 2 mL/min.

TABLE I

LIQUID-CHROMATOGRAPHIC EVALUATION OF STANDARD SUGARS AND MANNITOL[a]

Compound	*Retention time (t_R, min)*	*Capacity factor (k^1)*	*Relative response*
Psicose	5.75	2,14	0.988
Fructose	9.00	3.91	1.000
Mannose	11.40	5.23	0.404
Glucose	12.53	5.85	0.526
Mannitol	13.20	6.21	0.909

[a]Chromatographic conditions are described in the Experimental section.

TABLE II

YIELDS (%) FROM ISOMERIZATION OF D-FRUCTOSE WITH VARIOUS BASES[a]

Base	*Psicose*	*Fructose*	*Mannose*	*Glucose*	*Total*
Sodium hydroxide	6.0	26.2	3.6	21.4	57.2
Calcium hydroxide	5.7	26.2	2.4	17.1	51.4
N,N-Dicyclohexylcarbodiimide	8.4	14.4	2.0	8.8	33.6
Pyridine	12.4	25.8	5.1	6.4	49.7
Methanolic triethylamine	10.6	60.5	2.2	12.4	85.7
Aluminate resin	3.7	10.1	1.0	9.0	23.8

[a]Conditions of isomerization with each base are described in the Experimental section.

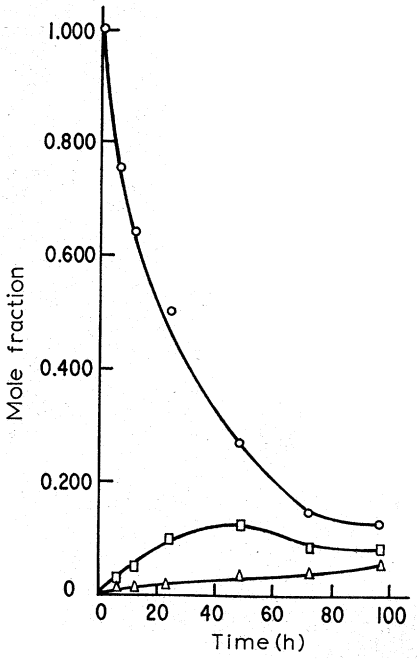

Fig. 2. Mole fractions of fructose (○), psicose (□), and glucose (△) produced as a function of time by refluxing (117°) 10% solution of D-fructose in pyridine.

Fig. 2 illustrates the accumulation in pyridine of psicose and glucose at the expense of fructose. In this instance, a 10% solution of D-fructose in pyridine was boiled under reflux and, for l.c. evaluations, an aliquot was withdrawn, diluted with 1:1 acetonitrile–water to 1.0 mg solute/20 μL solvent, and injected onto the column. The level of fructose decreased rapidly, with psicose increasing to a maximum at about 48 h. Glucose then continued to increase as the level of psicose fell off. After 96 h, 75% of the starting fructose had been converted, mainly to non-hexose products. When the isomerization was conducted by boiling higher concentrations of fructose in pyridine under reflux, the relative levels of the three hexoses were similar to those indicated in Fig. 2, but that level was reached earlier with increased concentration. For example, the sugar levels reached after 48 h in Fig. 2 were reached in just 2 h when a solution containing D-fructose at a concentration of 1.0 g/mL pyridine was boiled under refluxed. This rate enhancement was probably due to the increased reflux temperature (130 vs. 117°) at higher concentrations of D-fructose. As a result, the reaction time was decreased, less pyridine needed to be removed in the isolation, and the 2 h, 1 g/mL conditions were then routinely used.

Isomerization of D-fructose with sodium hydroxide and calcium hydroxide gave similar results, with inadequate yields of psicose. Treatment with the aluminate resin and with aqueous triethylamine resulted in extensive fragmentation of fructose and low yields of hexoses, including psicose. With methanolic triethylamine, l.c.

peaks appeared that interfered with psicose, but psicose quantitation was possible. Isomerization with sodium aluminate in solution (rather than resin-bound) gave only traces of psicose and glucose under the conditions tested, and most of the fructose could be recovered. Interestingly, whereas the equilibria in the alkali bases (sodium and calcium hydroxides) favor glucose over psicose by ~3:1, this ratio is greatly decreased in the nitrogenous bases *N*,*N*-dicyclohexylcarbodiimide and pyridine. Obviously, associations between the various bases and the product sugars control the position of the equilibrium. The conditions used for the isomerization of fructose by *N*,*N*-dicyclohexylcarbodiimide were precisely as described earlier by Passeron and Recondo[11], and the ratios of the hexoses found in this study were the same. In their report however, the hexoses accounted for all of the D-fructose consumed, and 3-fold increases in yields were reported, with psicose in 25% yields. The present study does not corroborate their findings, and most of the fructose was degraded.

As the most effective way to produce psicose in mixtures was to boil concentrated solutions of D-fructose in pyridine for short times under reflux, efforts were directed towards isolating D-psicose from these mixtures.

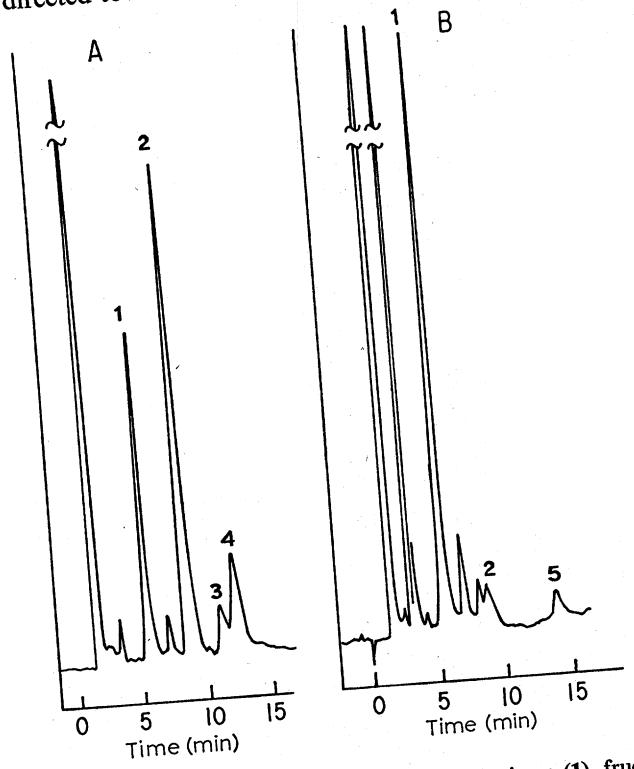

Fig. 3. Liquid-chromatographic profiles of psicose (**1**), fructose (**2**), mannose (**3**), glucose (**4**), and mannitol (**5**) in the isomerized mixture before (A) and after (B) fermentation by yeast. Chromatogram A represents the mixture produced by boiling D-fructose at 1 g/mL concentration in pyridine under reflux.

TABLE III

HEXOSE COMPOSITION (%) OF SYRUPS RESULTING FROM ISOMERIZATION OF D-FRUCTOSE[a], FOLLOWED BY FERMENTATION[b]

	Psicose	*Fructose*	*Mannose*	*Glucose*	*Mannitol*
Isomerization	11.9	32.2	5.5	6.0	0.0
Fermentation	28.0	1.7	0.0	0.0	1.5

[a]Fructose in pyridine (1 g/mL) was refluxed for 2 h. [b]The fermentation procedure is described in the Experimental section.

The early work of Zerban and Sattler[1] demonstrated that psicose is not fermented by bakers' yeast. The primary advantage in using a fermentation step to remove hexoses other than psicose from the isomerized mixtures was that the fermentate contained a much higher level of psicose/g of syrup than did the isomerized syrup before fermentation. Thus, smaller cellulose columns could be used in the final chromatographic step. Fig. 3 shows chromatograms of isomerized mixtures before and after fermentation with yeast. It may be seen that fermentation removes all of the glucose and mannose, and most of the fructose. A product of low retention-time (3.33 min) present as a trace in the isomerized mixture was greatly increased by fermentation. Another product (t_R 7.14 min) was present in small amounts both before and after treatment with yeast, and another (t_R 8.33 min), eluted just before fructose, resulted from fermentation. Thus, aside from the enrichment in psicose, the final chromatographic step for isolation of psicose was not otherwise simplified by fermentation. The traces of fructose remaining, the mannitol produced, and possibly other unidentified products produced by yeast, have R_F values in t.l.c. close to that of psicose. Table III summarizes the yields of hexose from isomerization of D-fructose before and after fermentation.

Preliminary experiments were conducted to establish whether direct acetylation of the isomerized mixture in pyridine would facilitate chromatographic isolation of psicose on silica gel, as its crystalline pentaacetate. Unfortunately, deacetylation of psicose pentaacetate with sodium methoxide appeared to isomerize the resulting free sugar rapidly to fructose, glucose, and mannose.

EXPERIMENTAL

General methods. — Sugars in isomerized mixtures were quantified by peak-height measurement after separation by high-pressure liquid chromatography (l.c.) on a Waters Associates μBondapak/Carbohydrate column. Peak heights for sugars were compared with those of corresponding sugars in standard solutions. The modular chromatographic system included an Instrumentation Specialties Co. (ISCO) metering pump model 314, series 1240-003, a pressure monitor (ISCO model 1590), and a

Waters Associates model R401 refractive-index detector. The eluting solvent was 88:12 (w/w) acetonitrile–water flowing at 2 mL/min, and the strip-chart recorder (Linear Instruments Corp.) was set at 18.2 cm/h. Normally, a total of 1.0 mg solute/20 μL was injected onto the column by using a loop injector (Altex Scientific Inc.). T.l.c. was performed on Eastman cellulose sheets (no. 13255) eluted with 8:6:3:3 *tert*-butanol–butanone–88% formic acid–water (solvent A) and spots were made visible with a spray consisting of diphenylamine hydrochloride (0.5 g), aniline (0.5 mL), acetone (25 mL), and 85% phosphoric acid (2.5 mL). Color developed after the plate had been placed for 10 min in an oven at 95°. Column chromatography with cellulose powder (Whatman no. 3199-u) used as the adsorbent and 10:8:1:1 *tert*-butanol–butanone–88% formic acid–water (solvent B) as the eluant. Column fractions were monitored by t.l.c. as already described.

Conditions for isomerization of D-*fructose.* — Various bases were evaluated for their efficiency in converting D-fructose to D-psicose.

A. Sodium and calcium hydroxide. A 10% solution of D-fructose in each base (0.035M) was stirred for 7 days at room temperature, made neutral with Dowex-50 (H^+), and filtered; the filtrates were evaporated to syrups.

B. Aluminate. The aluminate form[14] (10 g) of Bio-Rad AG 1-X8 (200–400 mesh) as the moist resin was added to a solution of D-fructose (1.00 g) in 10 mL of water. The mixture was stirred for 18 h at 40°, filtered, neutralized with Dowex-50 (H^+) resin, again filtered, and then evaporated to a syrup. Solution isomerization with sodium aluminate was conducted by adding D-fructose (1.0 g/eq) to 15 mL of M sodium aluminate (3 eq.) and stirring for 48 h at room temperature. The solution was adjusted to pH 6.8 with phosphoric acid, aluminum salts were removed by filtration, and the filtrate was evaporated to a syrup.

C. N,N-*Dicyclohexylcarbodiimide.* This isomerization was effected as described previously[11]. A solution of D-fructose (0.18 g) and *N,N*-dicyclohexylcarbodiimide (1.00 g) in methanol (10 mL) was heated in a sealed tube for 15 h at 85°. The reaction was quenched by adding water (10 mL), extracted with ether to remove reagents and colored products, and the aqueous layer was evaporated to a syrup.

D. Triethylamine. D-Fructose (1.00 g) was stirred in both 5% aqueous and methanolic triethylamine (20 mL) at 60°. Portions from each reaction were taken at 6 and 18 h and evaporated to syrups.

E. Pyridine. After early recognition that this is the most effective and convenient base, it was examined under various reaction-conditions. These are described in Results and Discussion. The same isolation procedure was used, regardless of the experiment. At the completion of a given reaction, pyridine was removed by concentration with the aid of toluene. The resulting, dark syrup was dissolved in methanol (10 mL/g of starting fructose), decolorizing charcoal was added, the mixture was refluxed for 0.5 h, filtered through Celite, and concentrated to a syrup; water (5 mL/g starting D-fructose) was then added. The aqueous solution was extracted three times with equal volumes of dichloromethane to remove more color and hydrophobic products. The aqueous phase was withdrawn and evaporated to a syrup.

Fermentation of sugar mixtures. — This procedure is now described for the syrup (63.9 g) resulting from processing of the isomerization reaction of D-fructose (75.0 g) in pyridine (75 mL) under refluxing conditions for 2 h. The syrup was dissolved in water (400 mL) and transferred to a 4-L Erlenmeyer flask. A suspension of bakers' yeast (84 g) that had been stirred into 400 mL of warm (35°) water was added, and the mixture was stirred for 12 h, during which time the flask was gently aerated, and then filtered through Celite and stirred with Bio-Rad AG 11A8 zwitterion resin (50–100 mesh). Ionic materials as well as the yeasty aroma were removed by this treatment. The mixture was filtered and concentrated to a syrup. Ethanol (500 mL) and decolorizing charcoal were added, and the mixture was boiled for 0.5 h under reflux and filtered through Celite; an orange solution resulted, which was evaporated to a syrup (21.5 g).

Chromatographic isolation and characterization of psicose. — After slurrying 300 g of cellulose powder in solvent B, it was packed into a glass column (42 × 500 mm). To the column was added a solution of 6.0 g of the fermented mixture in 6 mL of methanol. The scale of the fractionation may be modified, but a 50:1 ratio of absorbent to syrup is recommended. The column was eluted with solvent B, and progress monitored by t.l.c. on cellulose with solvent A. A material having R_F 0.670 was eluted first, and then psicose was eluted (R_F 0.392). The fractions containing only psicose were combined and concentrated to a brownish syrup (1.40 g). Decolorizing with charcoal yielded a yellow syrup that co-chromatographed with authentic psicose (l.c., t.l.c. of psicose and g.l.c. of the trimethylsilylated derivative). D-*ribo*-Hexulose phenylosazone was prepared in quantitative yield as previously described[11] and, after one recrystallization, had m.p. 162–163°, in agreement with the literature value[11].

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